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Turn Mimetics for Peptide Design

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Peptides play an important role in the regulation of a wide variety of biological functions, acting as hormones, neurotransmitters or inhibitors. Unfortunately, the therapeutic use of synthetic peptides is often hampered by their lack of metabolic stability and their inadequate transport properties. Substantial evidence exists that many peptides adopt a β-turn conformation in their active, receptor-bound form. A replacement of these turns by peptidomimetics can be beneficial to the stability and other properties as compared to the natural peptide. The conformation of a β-turn can be stabilized either by modifications that influence the conformational behaviour of the peptide backbone or side chain (peptide surrogates) or by templates used as a substitute for the β-turn skeleton. In this summary, we present the variety of organic template molecules for β-turn mimics based on a thorough search in the recent literature.

Contrary to our expectations, only 37 β-turn mimetics were found with a literature search in *Chemical Abstracts* up to June 1996 (Fig. 1). The numbering of the templates corresponds to the reference numbering.[1–37] To limit the number of citations, only the most recent publications are given for the templates and, out of many interesting articles concerning β-turns, only a few were chosen.[38–42] The templates are analysed with respect to β-turn classification, activity and structure elucidation via X-ray crystallography, NMR spectroscopy or modelling studies. Work is under way to incorporate the mimetics as easy-to-use building blocks in the library of our modelling software.

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One type of loop observed in proteins is the β- or reverse turn (sometimes called β-bend) which changes the direction of two secondary structural elements. The β-turn is defined in terms of four consecutive residues, named i to $i + 3$, with the following properties: (1) these residues do not form a helix and (2) the distance between the Cα atoms of residues i and $i + 3$ is less than 7 Å. There are seven commonly used categories of β-turn classified by the main-chain torsion angles ϕ and ψ of residues $i + 1$ and $i + 2$. The most frequently occurring turns are of types I or II. They are related by a 180° flip of the peptide group between residues $i + 1$ and $i + 2$. Types I′ and II′ are the backbone mirror images of I and II. Each turn type has specific amino acid residue preferences for at least some of the positions because of the stabilizing contribution these residues provide.

We have classified the β-turn mimetics with respect to the turn type they are designed to mimic. One-third of all compounds are proposed to adopt a II or II′ turn type (**1**, **5**, **7**, **8**, **12**, **16**, **21**, **23**, **31**, **32**, **36**) and three are designed to mimic a I or I′ turn (**13**, **28**, **37**). All other compounds can either substitute for several kinds of turn or are intended to achieve a chain reversal. The templates can be categorized as internal or external β-turn mimetics according to the positions of the template atoms, i.e. if they lie within or outside the β-turn skeleton. The majority of compounds (22 templates) are external β-turns, the others resemble either internal mimetics (**9**, **13–15**, **21**, **30–37**) or are difficult to classify (**6**, **25**). We currently analyse the compounds with molecular mechanics and dynamics techniques (Insight & Discover Software, MSI Inc.) on a Siemens supercomputer S200. We investigated, for example, the influence of the stereochemistry of compound **24** at position 3 of the benzodiazepine ring on the proposed turn type (I or II′). One isomer of **24** is a potent inhibitor of the protein farnesyltransferase with an IC_{50} of 0·9 nM.[24] Molecular dynamics revealed that on average only the *S*-isomer of **24** forms a stable β-turn, which, however, does not belong to any of the turn classes described in the literature.

For almost one-third of the suggested turn mimetics no biological data have been published. Fifteen compounds (**1**, **2**, **5**, **8**, **12–14**, **21**, **22**, **24**, **26**, **28**, **29**, **31**, **34**) have been incorporated into potent ligands with a biological activity in the nM range. Eight compounds turned out to be weak or inactive ligands (**6**, **7**, **9**, **15**, **30**, **33**, **36**, **37**). The conformations of most of the compounds have been confirmed by X-ray structure determination (**1**, **9–11**, **16**, **19**, **21**, **23**, **28**, **29**, **35**), NMR spectroscopy (**3–6**, **12**, **13**, **17**, **20**, **22**, **26**, **27**, **30–32**, **34**, **36**) or modelling studies (**7**, **8**, **14**, **24**, **33**, **37**). Surprisingly, to the best of our knowledge, there is only one example (compound **28**) where the predicted and the experimentally determined structure of the protein (thrombin) in complex with the ligand was published.[28]

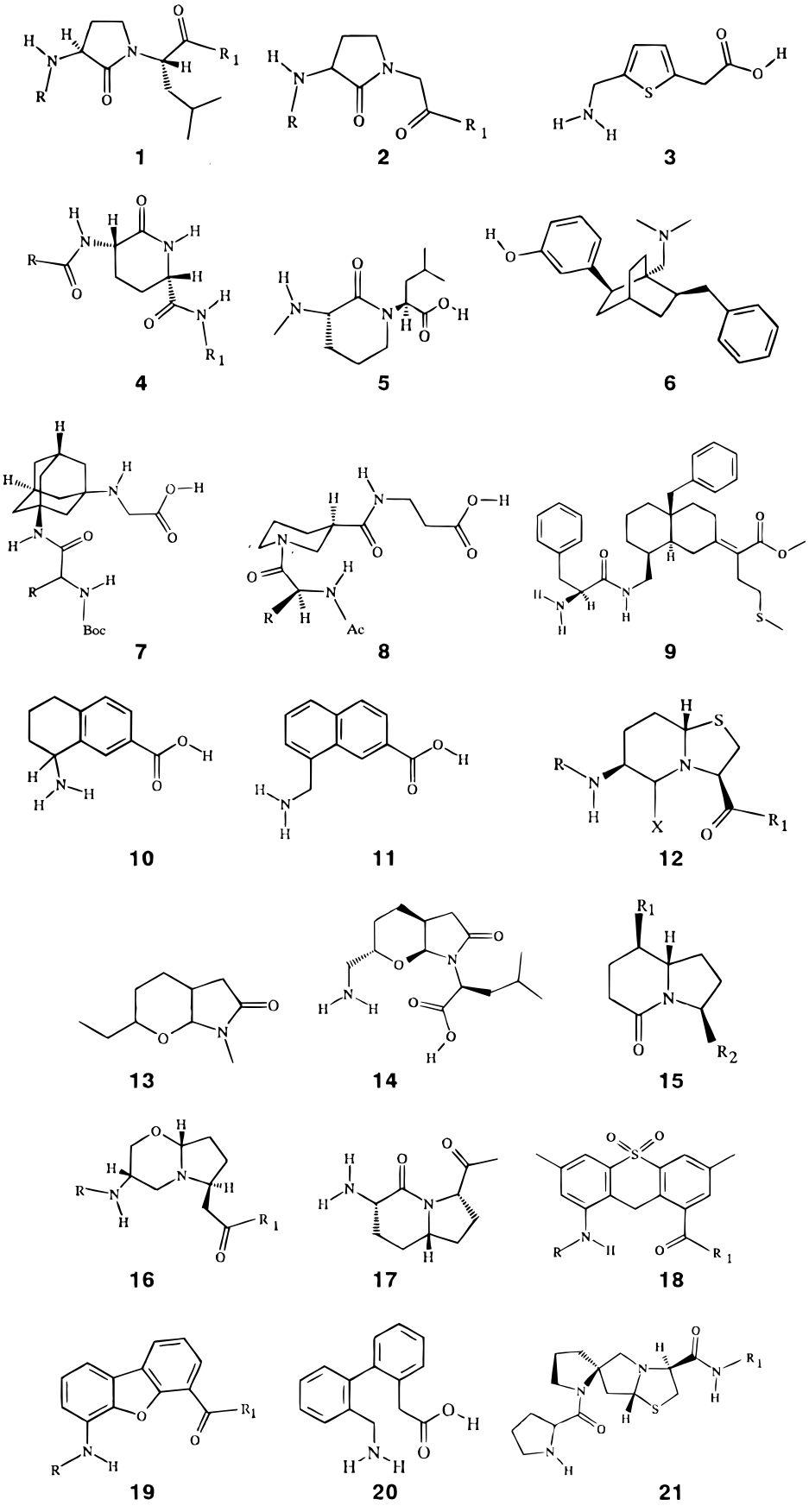

Fig. 1. Structural cartoon of the β-turn templates **1**–**37**.

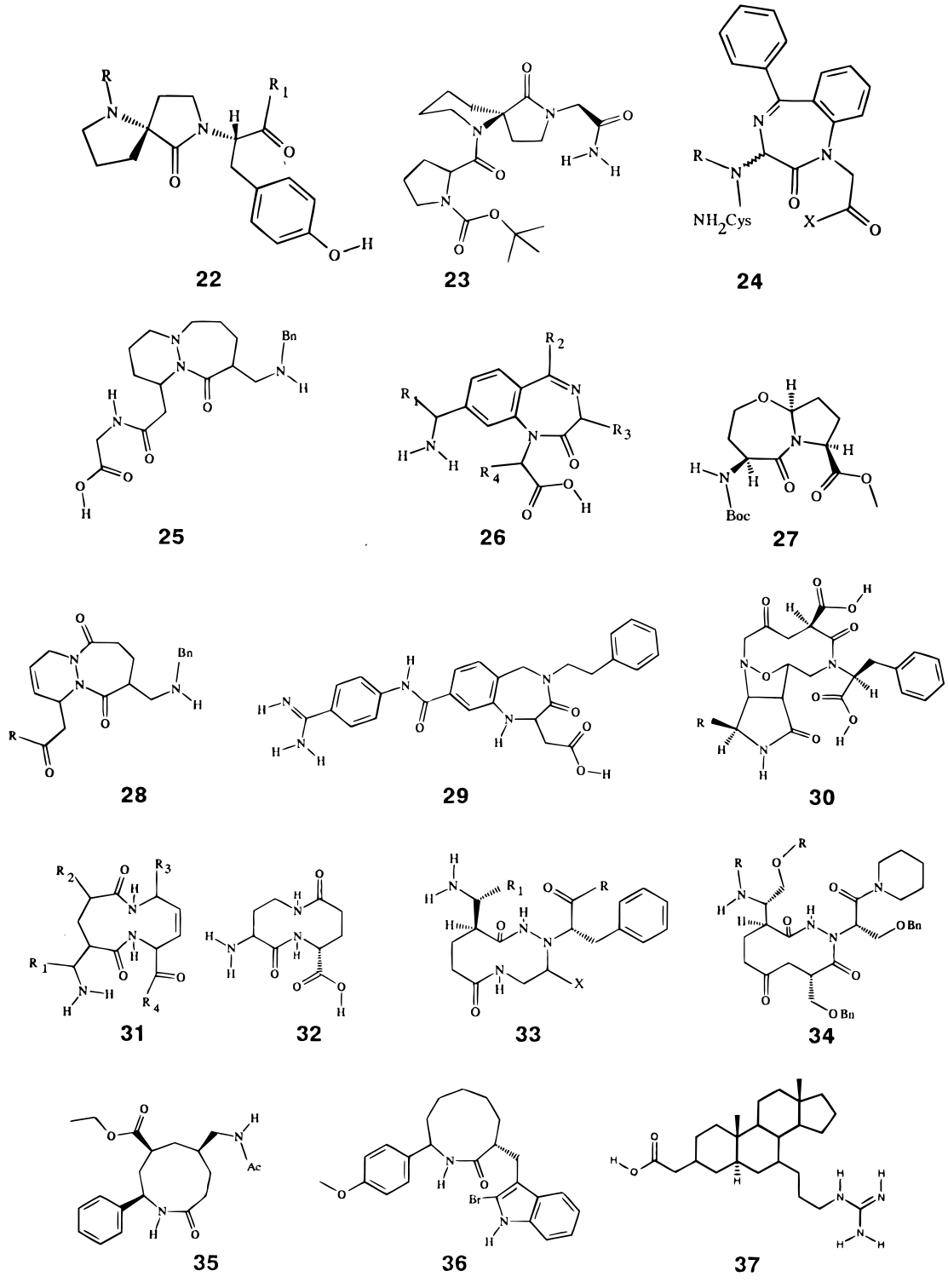

Fig. 1.—*Continued*

A superposition of the predicted and experimentally determined structures of compound **28** (R = Gly–Val–Arg–Gly–NH_2) showed that the positions of the bicyclic ring systems differ considerably, although—with respect to the peptide part of the mimetic—the predictions were in close agreement with the experiment. The conformation of **28** bound to thrombin displays a new binding mode in the active site of thrombin. This example stresses the importance of experimentally determined structures.

The importance of peptidomimetics for peptide research is not questioned. However, only a few β-turn mimetics have been published and even fewer have been characterized with respect to the three-dimensional

structure, the potency and the binding mode. Moreover, the application of some of these compounds is restricted due to a time-consuming or difficult synthesis. With the database of β-turn mimetics, we have an easy-to-use system at hand that will facilitate peptide modelling. Nevertheless, more experimental effort is needed to determine the three-dimensional structures and to assess the applicability of peptidomimetics in biological systems.

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Calmodulin Antagonists as Potential Antifungal Agents

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The ubiquitous proteins, the calmodulins (CAMs), play a key role in cellular proliferation.[1] They are highly conserved in most higher eukaryotes, but there is significant divergence in fungal CAMs, compared with those from other species, raising the possibility that these might be targets for novel antimycotic drugs.[2]

There are a few reports of the inhibitory effects of antifungal agents on calmodulin-mediated systems. It has been shown[3] that chloraniformethan inhibits CAM-dependent cyclic nucleotide phosphodiesterase (PDE), and the clinically useful azole antifungal ketoconazole at low micromolar concentrations inhibits this enzyme[4,5] and also CAM-dependent nitric oxide synthase.[6] To our knowledge, however, there have been no attempts to develop calmodulin antagonists specifically as antifungal agents.

As lead compounds we used the naphthalenesulfonamides, introduced by Hidaka and Tamaka[7] and further developed by MacNeil *et al.*[8] They have the general formula $ArSO_2NH(CH_2)_nNH_2$, where Ar is a 1- or 2-naphthyl residue, preferably halogenated and n lies between 6 and 10 for maximum inhibitory potency. Examples are W7 and J8 (Fig. 1). In our initial attempts to modify these compounds, the sulfonamide linkage in W7 was replaced by polar amide, urea, or thiourea groups. In each case the ability of the resulting compounds to inhibit CAM-dependent PDE was much reduced. However, substitution of the SO_2NH moiety by a *non-polar* ether or thio-ether linkage gave compounds equipotent with corresponding sulfonamides, and the ether group was adopted in all subsequent compounds synthesised.

Since W7 inhibits calcium-activated transglutaminases at concentrations similar to those required to bring about calmodulin antagonism,[9] the terminal side-chain primary amine necessary for this competing inhibition was replaced in our compounds by a number of tertiary amines. The most effective of these were found to be derived from pyrrolidine or piperidine, presumably because the pKa of *N*-alkylpyrrolidines (10·32) and piperidines (10·1) is close to that of primary alkylamines (10·64), whereas incorporation of imidazole (pKa 7·33) or morpholine (pKa 7·41) gave poorer antagonists. A similar substitution of pyrrolidine for the primary amino group has recently been found to enhance the CAM-inhibitory properties of some tamoxifen derivatives.[10]

In agreement with the earlier studies,[7,8] the potency as PDE inhibitors of our compounds, now with the general structure $Ar–O–(CH_2)_n–NR_2$, increased with the length of the alkyl side-chain. There was a marked rise at $n = 4$ to 6, with a further slight improvement up to $n = 9$. Although, in most of the compounds prepared, the aryl group was 1- or 2-naphthyl, 4-benzothiophenyl or 2-dibenzofuranyl residues were also effective. Interestingly, benzofuranyl-containing compounds were significantly less potent than analogous benzothiophenes. Representative results are shown in Table 1. The inhibition of CAM-dependent PDE was measured by the modified method of Thompson *et al.*[11] in which tritiated AMP liberated from cAMP was converted by snake venom to adenosine. The synthesis of the inhibitors, by standard methods, is described in a patent.[12]

The test organism selected for assaying antifungal activity in our compounds was *Pythium ultimum* Trow.

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